Methods – Supplemental Information

Serum extraction and cleanup. Extraction of AhR agonists from human serum was performed using a method largely from Murk et al (1997). All glassware was hexane-washed before use. Serum samples (2 ml) were aliquoted into a 16x100 mm glass centrifuge tubes. Two ml of methanol were added to the sample, and the mixture was vortexed. An additional 3 ml of hexane also was added and vortexed. Samples were centrifuged for 2 minutes at 1,500x g at RT. The hexane layer was transferred to a glass centrifuge tube. Four drops of 6M HCl were added to the remaining aqueous layer. The serum was then extracted 2x using 2 ml hexane each time, as above. All hexane extracts were combined. Finally, 0.5 ml of hexane was added to the aqueous phase and collected without mixing. All hexane extracts were combined, evaporated under nitrogen at 30^{o}C and dissolved in 0.3ml hexane: diethyl ether (97:3 V:V).

To prepare the acid silica column, silica (100 g) was prepared by baking at 200^{o}C for 24 hrs, cooled in a dessicator and mixed with 28 ml concentrated sulfuric acid. In an 8 mm drying column on top of a glass wool plug, 1.5 g of Na_2S0_4, acid silica to a height of 80mm and 1g Na_2SO_4 were added. The column was washed with 2x10 ml hexane: diethyl ether (97:3). The extract was applied to the column immediately and then eluted with 13ml hexane: diethyl ether (97:3). Samples were then evaporated under nitrogen at 30^{o}C until approximately 0.5 ml remained and quantitatively transferred to hexane-washed, tapered glass vial. After complete evaporation, samples were resuspended in 20 µl DMSO.

Serum charcoal treatment. Activated charcoal (0.25g, Sigma) was suspended in 50 ml PBS. A volume of charcoal in PBS equal to the volume of serum to be treated was added to a sterile micorcentrofuge tube. The charcoal was pelleted and all liquid was decanted. Serum was added to the pellet, vortexed and incubated at 45^{o} C for 1 hr. The slurry was spun at maximum

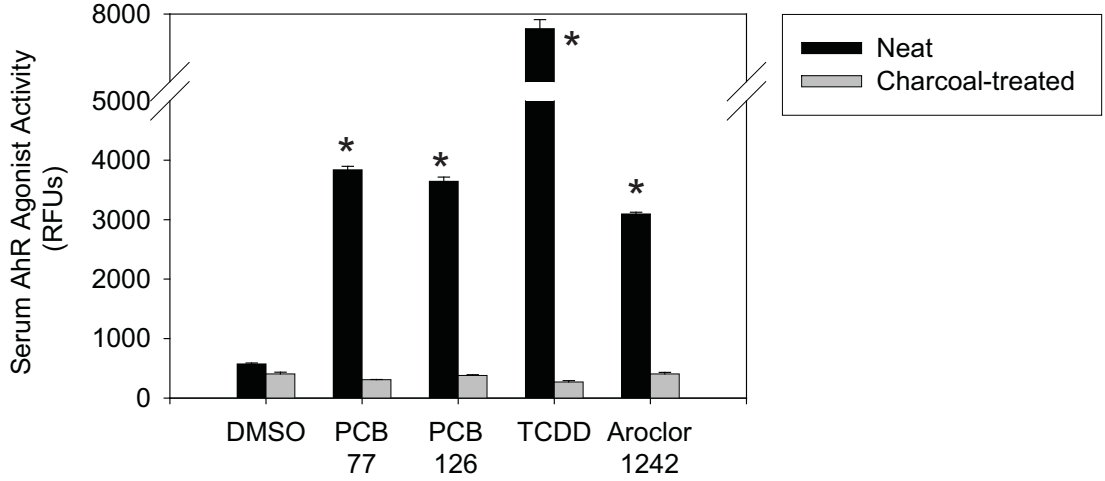
speed for 5 min. The supernatant was transferred to a new microcentrifuge tube and used in the serum AhR agonist activity assay.

In order to validate the efficiency of planar HAH removal, bovine serum (Hyclone) was supplemented with DMSO (1%) or known final concentrations of PCB 77 (10^{-6}M), PCB 126 (10^{-8}M), TCDD (10^{-9}M), or Aroclor 1242 (10^{-4}M)(Ultra Scientific). Aliquots of each were either left untreated or charcoal-treated, as described above. Serum AhR activity then was determined as described below using 10 μl of serum. Charcoal treatment of serum completely removed serum AhR activity induced by all of the agents (Supplemental Figure 1).

Assessment of AhR activity. H1G1.1c3 cells were maintained and prepared for experiments largely as described previously (Nagy et al. 2002). H1G1.1c3 cells were plated at a concentration of 7.5 x 10^4 cells per well in 200 μl of medium (αMEM, 10% FBS, 50 units/ml penicillin/streptomycin) containing G418 (968 mg/l). Cells were incubated for 24 hrs at 37˚C. Prior to addition of serum or serum extracts, the medium was replaced with 100 μl of medium without G418. Neat sera (10 μl) from experimental mice or from the Faroe Islands cohorts were applied to triplicate wells. For commercial pooled AB serum samples, four aliquots of each were prepared, and neat serum (10 μl) from each of the aliquots was applied to triplicate wells. Serum extracts (1 μl) were applied to triplicate wells. Each plate also contained a standard curve of H1G1.1c3 cells treated with 10^{-14}-10^{-9} M TCDD. In specificity experiments, wells were pre-treated with either DMSO (0.5% final concentration) or the AhR antagonist CH223191 (10^{-5} M final concentration)(EMD Chemicals, San Diego, CA) for 30 min. At least two wells were left untreated per plate. The plates were incubated at 33˚ C for 24 hrs. The plates were read in a fluorometic plate reader (Cytofluor 4000, Applied Biosystems or Synergy2, BioTek). The excitation wavelength was 485nm (20 nm bandwidth), and the emission wavelength was 530 nm

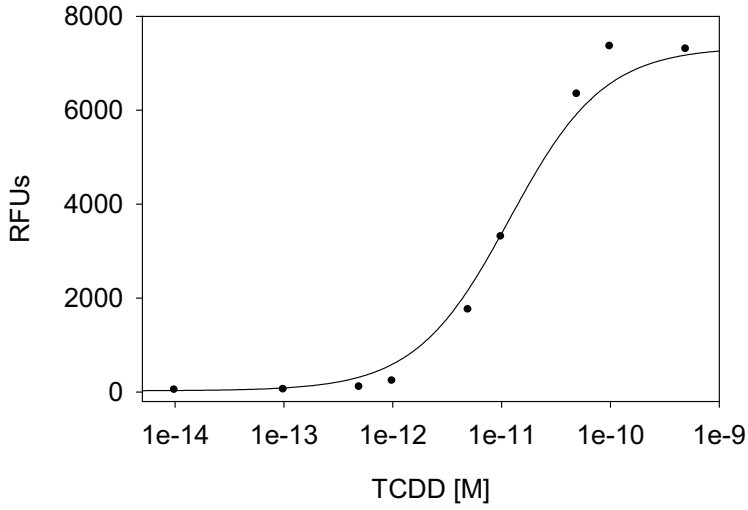
(25 nm bandwidth). Fluorescence in all experimental wells was normalized by subtracting the fluorescence measured in wells with untreated cells. All samples were analyzed in a continuous run to maximize comparability, with the exception of Figure 5 and Supplemental Figure 1 in which the samples in a given panel were analyzed at the same time.

For calculation of serum AhR agonist activity, the standard curve was fitted using the four parameter sigmoid Hill equation. Triplicate fluorescence measurements for each serum sample were averaged and used to determine the serum AhR agonist activity, in pg TCDD equivalents (eq)/ml, by interpolation from the fitted standard curve (Supplemental Figure 2).



Supplemental Figure 1. Charcoal treatment completely removes AhR activity from sera supplemented with known concentrations of HAHs. Bovine serum was supplemented with DMSO (1%), PCB 77 (10^{-6} M), PCB 126 (10^{-8} M), TCDD (10^{-9}M), or Aroclor 1242 (10^{-4}M). Aliquots of each were either left untreated or charcoal treated. Data are presented as means ± SE from 3 separately prepared aliquots. * - Significantly different from DMSO ($p<0.05$, ANOVA, Tukey-Kramer).

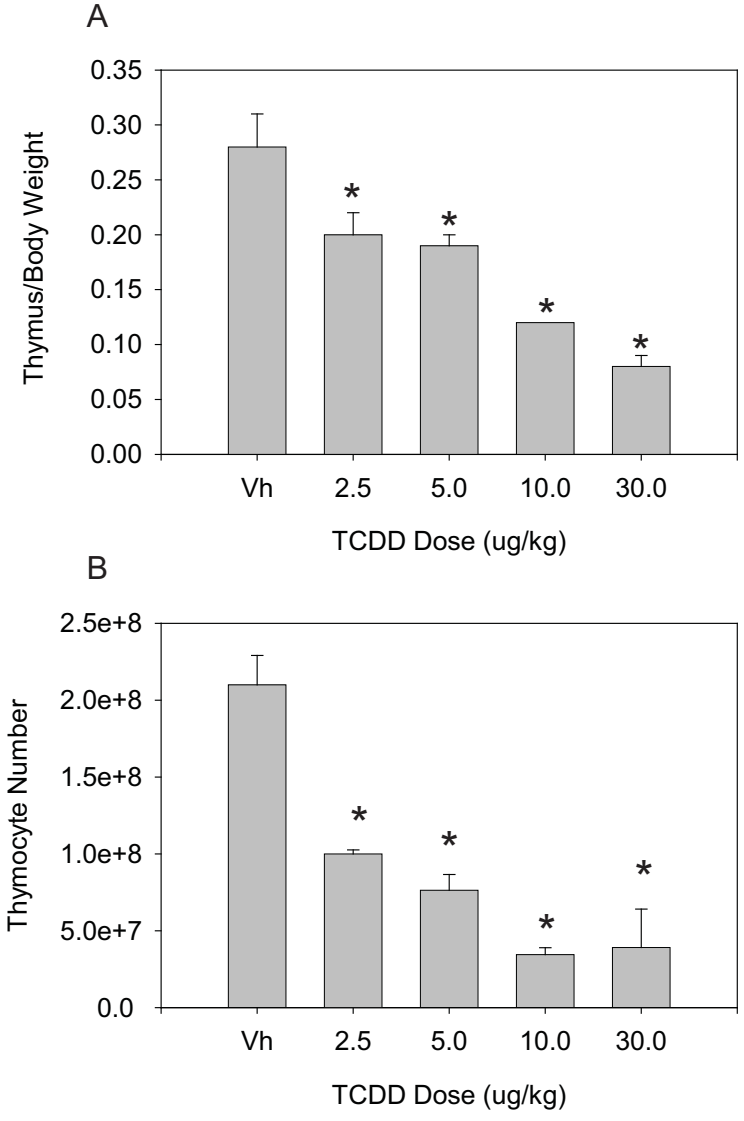
A



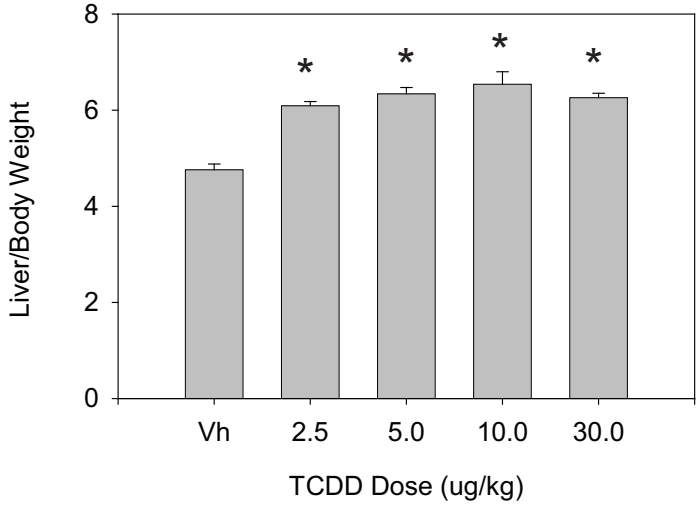
B

$$\text{AhR Agonist Activity [M]} = \left(\frac{c^b(y_0 - y)}{a + y_0 - y} \right)^{1/b}$$

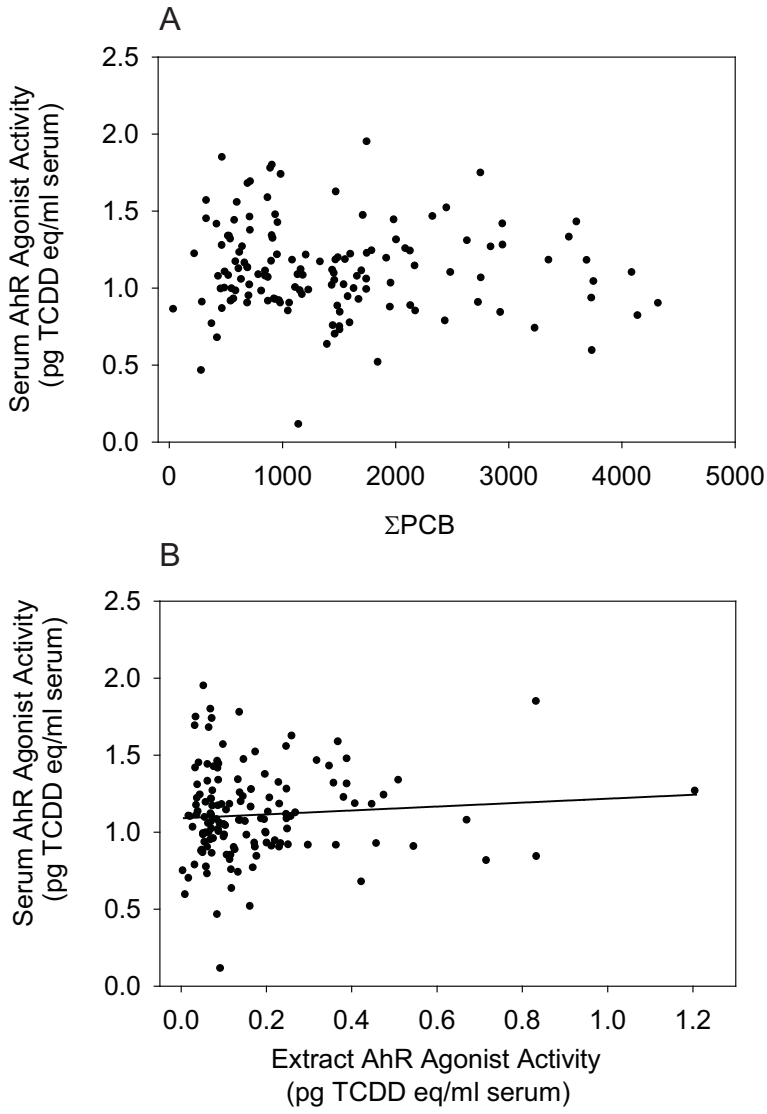
Supplemental Figure 2. Typical TCDD standard curve in H1G1.1c3 cells (A) and Hill equation used for data interpolation (B). a = RFU maximum, b = Hill coefficient, c = EC_{50}, y = measured RFUs and y_0 = RFU minimum.



Supplemental Figure 3. TCDD treatment results in reduced thymus weight (A) and thymocyte number (B). Thymus weights were normalized by dividing by the total body weight of the animal. Data are presented as means ± SE from 3-9 animals.* - Significantly different from vehicle ($p<0.05$, ANOVA, Dunnett's).



Supplemental Figure 4. TCDD treatment results in hepatomegaly. Liver weights were normalized by dividing by the total body weight of the animal. Data are presented as means ± SE from 3-9 animals. * - Significantly different from vehicle ($p<0.05$, ANOVA, Dunnett's).



Supplemental Figure 5. Lack of correlation between serum AhR agonist activity and ΣPCB (A) and AhR agonist activity in serum extracts (B). Data from samples in which all three analyses were performed are included.